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ABSTRACTS

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Analytical Methods and Procedures for Metals

Trace Element Analysis with Characteristic X-Rays Excited by Fast Cl^{n+} Ions.

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The present observations were made during the course of a study of a rare x-ray phenomenon, i.e., the decay of atoms in states with two K electrons missing by the emission of single photons with approximately twice the normal K_α energy ($2e-1\gamma$ transitions). These studies required relatively pure targets consisting of ~50–150 $\mu g/cm^2$ films evaporated on to thin carbon backings ~20 $\mu g/cm^2$ thick. For such experiments traces of contaminant elements can be detected and may interfere with the study of $2e-1\gamma$ transitions.

We show an example where ~100 ppm of Fe was detected in Ti, and a similar example where trace amounts of Br are detected in a thin target of KCl evaporated from single crystal KCl; and finally a case where a $2e-1_\gamma$ transition arising from the Cl^{n+} projectile is detected at the same level as trace amounts of Cu and Zn in a thin (~20 $\mu g/cm^2$) C foil. Typical of the data presented here are those for 70 MeV Cl^{9+} on Ti, where the Fe impurity was found. In this case a beam of 8 na supplied by the TUNL FN tandem Van de Graaff was incident on the target for 1.8 hr, and a 6-mil aluminum absorber reduced the counting rate in the x-ray detector to < 150 cps, to eliminate pile up and gain shifts. The x-ray detector was intrinsic germanium, 80 $mm^2 \times 5$ mm with a nominal resolution of 170 eV at 5.9 keV.

Impurity concentrations were estimated either by using our own previously measured cross-section data or by assuming that the x-ray production cross sections scaled inversely as the square of the K-shell binding energy. A significant problem in using Cl^{n+} beams is the energy shift of x-ray peaks due to multiple ionization with projectile energy. Various background effects and other experimental problems associated with the use of heavy-ion beams are also discussed.

Although we did not optimize our experiment for trace element analyis we would like to point out that K-shell electron removal cross sections do increase as Z^2 of the projectile for a given velocity, and so heavier ions should offer advantages over protons, especially for heavier elements, by reducing the irradiation period and/or beam intensity requirements.

Trace Metal Concentrations in Plants and Soils By Photon-Induced X-Ray Emission Analysis (PIXEA).

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During the past two years we have proceeded to develop a proton-induced x-ray emission analysis (PIXEA) system for quantitative analyses of biological and environmental samples. The method employs a 3-MeV proton beam to excite characteristic x-ray emission from the irradiated sample. Typical sensitivities for the PIXEA system are 1–100 ppm (dry weight) for most elements in the region between Cl ($Z=17$) and Pb ($Z=82$).

One project undertaken by this laboratory involved a series of studies utilizing PIXEA to determine metal abundances in Ribwort Plantain and surrounding soil. These studies were prompted by the following questions: (1) How do metal abundances in soil vary with depth and with distance from a pollutant sources? (2) To what extent is the metal content of soils reflected in the metal abundances of plants grown in these soils? (3) What fluctuations in metal concentrations are to be expected between different leaves of the same plant? Within a single leaf?

Three experimental sites were chosen for the study: site 1 is adjacent to a busy highway; site 3 is located 75 m distant in an open, partially wooded area; Site 2 is intermediate between sites 1 and 3. Only the results from sites 1 and 3 are presented here. Abundances are reported for 9–16 elements representing analyses of approximately 80 samples from sites 1 and 3, including leaves, soil pellets, and soil extracts. In addition, 18 regions of a single leaf were analyzed to determine intraleaf concentration variations.

Our results confirm the existence of high abundances for some heavy elements for the roadside site and the concentration falloff for most elements with distance from the highway. In addition, metal uptake in plant leaves is shown to have a strong dependence on leaf age. Concentration fluctuations within a single leaf are not significant enough to obscure these general trends.

Trace Elements in Diseased and Normal Lung by Use of PIXEA and Other Techniques.

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We have carried out multielement analyses of lung autopsy specimens and lung lavage materials using proton-induced, x-ray emission analysis (PIXEA). Current sensitivities with this technique extend to 0.1 ppm for some heavy metals. Determinations of Cd in these samples were made by using flameless atomic absorption which was sensitive to 1 ppb. Lung materials were examined by optical microscopy for inorganic particles. Other tools used were electron microscopy, electron microprobe spectrometry and scanning Auger electron spectroscopy. Our immediate aim was to establish trace element abundances in normal and abnormal lung tissues and materials. In addition, our longer-range goals are the development of preparation methods for biological and environmental samples which involve a minimum of chemistry, application of statistical methods for finding correlations among samples with complex compositions, and development of valid criteria for detectability. We make a plea that workers in the field of trace element analysis cooperate to establish generally accepted methods for determining and reporting detectability limits.

Dietary, Nutritional, and Developmental Factors in Metal Intoxication

Trace Element Interactions in Mammalian Cell Culture.

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Trace element interactions previously studied in whole animals in this laboratory and elsewhere were investigated in mammalian cells in culture. The Chang's liver cell line and the 3T3 cell line were used in growth and radioisotope uptake studies. These experiments were designed to assess the potential of a tissue culture model for the elucidation of trace element interactions *in vitro* as compared to *in vivo*.

Results of experiments utilizing these cell lines for five and six day growth periods revealed that both cell lines were: protected against selenite toxicity by mercuric chloride and monomethyl mercury and protected against mercuric chloride toxicity by selenite. Radioisotope uptake studies were conducted with ^{203}Hg and ^{75}Se during a 6-day growth period in Chang's liver cells. These experiments showed that: selenite increased mercuric chloride uptake, methylmercury inhibited selenite uptake, and mercuric chloride did not affect the uptake of selenite. The molar ratio of Hg/Se which provides optimal protection under various conditions was also investigated.

By using confluent nondividing cultures of 3T3 cells, 2-hr uptake studies were conducted with ^{65}Zn. Copper (II), silver (I) and mercury (II) did not affect ^{65}Zn uptake in 3T3 cells. Cadmium, on the other hand, inhibited ^{65}Zn uptake in 3T3 cells and this inhibition was dependent upon the presence of amino acids in the uptake medium. The uptake of ^{65}Zn by 3T3 cells exhibits a saturation kinetics pattern from 0.6 to 5.0 μM zinc chloride which is affected by the presence of amino acids and/or serum in the medium. In a similar 2-hr uptake study with ^{64}Cu in dividing Chang's liver cells, mercuric chloride increased ^{64}Cu uptake by the cells.

Effects of Copper on Marine Fish Eggs and Larvae.

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Since copper is one of the most ubiquitous trace metals in the marine environment and has been shown to be highly toxic to aquatic organisms, a series of experiments was designed to compare the sensitivities of

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larval fish, and determine the effects cupric ion on spot eggs and larvae. Using larval pinfish, *Lagodon rhomboides*, as the test organism, we first demonstrated that the flow-through system was superior to the static exposure system because it gave more uniform total element concentrations. By using the flow-through system, interspecies comparisons of copper toxicity were done over a period of 14 days, and the order of sensitivity on the basis of total copper concentration as shown by LC_{50}, was pinfish, *Lagodon rhomboides*, 0.15 ppm; spot, *Leiostomus xanthurus* 0.16 ppm; Atlantic croaker, *Micropogon undulatus*, 0.21 ppm; and Atlantic menhaden, *Brevoortia tyrannus*, 0.61 ppm. The measurements made of the effects of free cupric ion on spot eggs and larvae indicate that the eggs are more sensitive than the larvae. In addition the chelation of copper by tris (hydroxymethylamino) methane reduced the toxicity of copper to the larvae significantly. The 50% survival point for the eggs occurred at a cupric ion activity of $10^{-9}M$ and for the larvae at activities in the range of $10^{-8.1}$–$10^{-8.5}M$. Such values approach the estimated range for cupric ion activity in seawater.

Accumulation of Mercury in Fish Fed a Naturally High Mercury Diet. P. J. WHALING, R. T. BARBER, and J. C. PAUL, *Duke University Marine Laboratory, Beaufort, North Carolina 27516.*

Juvenile pinfish *(Lagodon rhombodies)* were fed high and low mercury diets while being held in running seawater at ambient summer temperatures for 160 days. The high mercury diet was blue marlin *(Makaria nigrians)* axial muscle containing 15 ppm (wet weight) mercury. The low-mercury diet was oyster *(Crassostrea virginica)* containing 0.1 ppm (wet weight) mercury. Differential uptake and accumulation of mercury in the fish fed ble marlin occurred in the axial muscle, liver, kidney, heart, and brain tissue of the pinfish. Cadmium, chromium, copper, iron, lead, manganese, and zinc decreased in the pinfish axial muscle. Deaths among the pinfish fed blue marlin started to occur on day 30 and continued at relatively even spaced intervals until the experiment was terminated on day 160. During this period there were no deaths in the control group of pinfish fed the low mercury diet. No selenium protection from mercury poisoning was observed in the case of marlin as a high mercury source. This differs from the results of others, who have found selenium protection when tuna is used as a high mercury food source.

Effects of Cerium and Platinum on the Behavior and Development of the Mouse. EDWARD J. MASSARO, CARL STINEMAN, and FRED C. OLSON, *Department of Biochemistry, SUNY at Buffalo, Buffalo, New York 14214*, and JOHN B. MORGANTI and BRADLEY A. LOWN, *Department of Psychology, State University College of Buffalo, Buffalo, New York 14222.*

Open-field activity (ambulations and rearings) and tissue/organ uptake were investigated in mice receiving cerium citrate, platinum sulfate, or sodium hexachloroplatinate at the 7 day LD_5 or LD_{25} levels via the IG or SC routes of administration. Observations were made at 4 hr, or 1, 3, or 7 days after administration.

For platinum sulfate via the IG route, anova of ambulations revealed significant main effects of time which were the result of decreased ambulations in the high platinum (Pt) groups at 4 hr and 7 days.

Anova of the ambulations data for the IG or SC cerium revealed significant main effects of route, dose, and time; significant interactions of route by time, dose py time and route by dose by time interaction. For rearings, route, dose, time and interactions of route by time and route by dose by time were significant. In all cases, the effects were one of decreased activity. Separate analysis for ambulations and rearings of the SC animals yielded significant main effects of time and dose and a significant interaction for ambulations. Via the SC route, the main effect of time was accounted for primarily by decreased activity associated with the high Ce dose. The time by dose interaction was accounted for largely by the fact that, at 4 hr, the animals receiving the high Ce dose were significantly less active than those at all other doses and times. The balance of the interaction could be accounted for by the fact that, at 4 hr, the low Ce animals were less active than controls, and that, as 1 day, the high Ce animals were less active than controls. Analysis of the IG route yielded no significant effects on ambulations or rearings.

Anova of the effects of Ce (IG, SC) on "hole-in-the-board" exploratory behavior revealed significant main effects of time and route and significant interactions of time by route, time by dose and route by dose. The major source of the time and route effects and the time by route, time by dose, and route by dose interactions was the very depressed exploratory scores of the SC Ce groups at 4 hr. Anova of retention of passive avoidance learning by IG and SC Ce animals 24 hr after the initial training trial revealed significant main effects of time, dose and trials and significant interactions of time by dose, time by trial sand time by dose by trials.

Following Ce administration (SC at the LD_1 level) on gestational day 7 (G 7), both male and female pups (Ce × Ce cross-fostered) exhibited significantly depressed activity on day 7 but not on day 12 post partum (P 12). When Ce was administered on G 12, male pup activity was significantly decreased in the Ce × Ce and Ce × citrate animals on P 12. Ce administered to mothers on P 2 resulted in significant decreased activity of male pups on P 7 and 12. On P 12, the activity of female pups was increased significantly.

Following hexachloroplatinate administration on G 7, the activity of female saline × Pt pups was significantly increased on P 7. Pt administration on G 12 resulted in significantly decreased activity of both male and female Pt × saline pups on P 12. The activity of male Pt × Pt pups was decreased similarly, but not significantly (high variability). When Pt was administered to dams on P 2, male pup activity was increased significantly on P 12.

In general, administration of Ce or Pt produced weight decreases that were statistically significant, although of small percentage difference (maximally ~15%).

Assessment of Developmental Toxicity Associated with Chronic Lead Exposure. L. D. GRANT, C. A. KIMMEL, C. M. MARTINEZ-VARGAS, and G. L. WEST, *University of North Carolina, Chapel Hill, North Carolina 27514 and NIEHS, Research Triangle Park, North Carolina 27709.*

The effects of chronic lead exposure on prenatal and postnatal development in the rat were examined. Female rats were chronically exposed to lead acetate (PbAc) via their drinking water (0, 0.5, 5.0, 50, 250 ppm) from weaning through mating, gestation and lactation. Evaluation of lead effects on their growth and development of reproductive functions revealed that: vaginal opening was significantly delayed (1-2 weeks) in the females exposed to 50 and 250 ppm. Estrous cycles, however, appeared to be essentially normal, as did pregnancy rates when mated. Females in the 50 and 250 ppm groups were significantly lower in body weight at mating and parturition than control animals, although reductions in their food and water intake were not statistically significant.

Atomic absorption analyses of tissue lead at time of mating (7 weeks after weaning) demonstrated dose-dependent lead concentrations in blood and bone, with significant elevations for the 50 and 250 ppm females, e.g., blood Pb for 250 ppm group was approximately 60 μg%. Brain Pb, however, was not detectable except in the 50 and 250 ppm groups at that time and ranged from 0.1 to 0.35 ppm. Evaluation of Pb effects subsequent to mating showed that: 250 ppm Pb caused a small, but significant, increase in number of resorptions. No significant teratogenic effects were detected, however, in offspring of any treatment group. Animals of all treatment groups allowed to deliver their young had normal 21–22 day gestation periods and produced litters of normal numbers.

Postnatally, although mortality through weaning was not significantly increased by any Pb treatment, offspring from the 50 and 250 ppm groups did exhibit delays in physical and behavioral development. Growth rate was slowed in these groups, with offspring of these groups weighing significantly less than control animals at weaning. Delays in physical development for these animals were also apparent (e.g., delayed time of vaginal opening). As for behavioral development, delays in the 250 ppm group were observed in the appearance of righting reflexes and in the maturation of locomotor patterns from "pivoting" to crawling and walking. No significant effects on open-field activity levels were observed, however, at 21, 24, 27, or 30 days of postnatal age. In summary, chronic Pb exposures at levels producing high body burdens did not appear to markedly affect prenatal development, but did have significant effects on postnatal development.

Effects of Chronic Lead Administration on Schedule-Controlled Behavior of Pigeons. G. T. BARTHALMUS, *Department of Zoology, North Carolina State University, Raleigh, North Carolina 27607,* J. D. LEANDER, and D. E. MCMILLAN, *Department of Pharmacology,* and P. MUSHAK and M. R. KRIGMAN, *Department of Pathology, School of Medicine, University of North Carolina, Chapel Hill, North Carolina 27514.*

Pigeons were trained to peck a response key under a multiple fixed-ratio 30 response (FR 30) fixed-interval 5 min (FI 5) schedule of food presentation. When rates of responding stabilized, lead acetate (6.25, 12.5, and 25 mg/kg) or sodium acetate solutions were given daily by gastric intubation. Blood lead determinations were measured weekly by atomic absorption spectrometry and responding under the multiple schedule was measured daily. Control injections of sodium acetate did not affect responding under either component of the schedule. The 25 mg/kg dose of lead acetate decreased rates of responding after 3 to 10 days and usually was lethal between 18 and 35 days. Postmortem examination of the birds showed obvious esophageal atonia, damage to the crop, weight loss, and cerebral hemorrhage. When administration of the 25 mg/kg dose was discontinued in the surviving birds, responding gradually began to recover, but rates of responding remained low and highly variable under both components of the schedule. Daily administration of the 12.5 mg/kg dose of lead acetate caused one death, while obvious gross symptoms did not occur in the other birds. However, 12.5 mg/kg of lead acetate decreased rates of responding under both schedule components within 30 days of intubation. When lead acetate administration was discontinued after responding had been almost completely eliminated, dramatic increases in rates of responding sometimes occurred before response rates restabilized at levels comparable to those before lead administration. The 6.25 mg/kg dose had little effect on rates of responding during 70 days of intubation. The weekly blood lead levels were very high during treatment with lead acetate (150 to 3470 μg/100 ml), but the correlation between blood lead level and behavioral change was low.

Acute Lead-Induced Vasculopathy in the Neonatal Rat. M. R. KRIGMAN, G. HARDY, C. BRASHEAR, and P. MUSHAK, *Department of Pathology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27514.*

The role of vascular changes in the genesis of lead encephalopathy has been stressed by a number of investigators. Vasculopathy may be a significant feature of lead intoxication produced in the suckling rat. When developing rats are intoxicated in a controlled manner, the interrelationship among dose, age, and severity of vasculopathy can be defined.

Neonatal rats were first intoxicated with a graded series of lead doses. Litters of 2-day-old rats were given one of 9 different doses of lead, 10–3000 μg/g body weight per day, via gastric intubation. Cerebral and spinal cord hemorrhages regularly developed within 24–48 hr in thos pups receiving 750 μg or more. When the effect of age was studied, 7 groups ranging from 2 to 60 days were treated. Rats intoxicated prior to 20

days of age regularly developed grossly discernible brain hemorrhages; 20-day-old rats showed microscopic hemorrhages. Hemorrhages were not observed in 30- and 60-day-old rats. Moreover, the younger the rat, the more rapid was the development of hemorrhages and the more severely affected was the cerebrum. Animals that survived the encephalopathy and were still being treated showed the residua of parenchymal hemorrhages but no new hemorrhages. This was also apparent in the histologic studies. Initially, segments of capillaries showed endothelial damage. With time there was regeneration, and an increased number of endothelial cells.

Any mechanism proposed for pathogenesis of the vasculopathy must be reconciled with the observations that not only are the vascular alterations related to the lead dosage but to the apparent state of vascular system development. In addition, the vascular changes are reversible in the face of a continued lead burden.

Inorganic Lead Affects Tyrosine Accumulation by Isolated Choroid Plexus but Not by Other Brain Regions.

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Lead nitrate is accumulated 70 times more strongly by choroid plexus than by neural tissues. This finding raises the possibility that selective choroidal dysfunction may play a role in the pathogenesis of lead encephalopathy. We have studied the effects of lead on the accumulation of tyrosine by isolated choroid and brain slices, a function which is known to be carrier-dependent.

Freshly excised cat tissues were preincubated for 15 min in artificial cerebrospinal fluid (CSF) only or in CSF with added lead nitrate ($5 \times 10^{-6} M$) and then incubated for 5 min with varying concentrations of L-tyrosine.

In the controls the transport rate $V_{max} \pm$ SEM and $K_m \pm$ SEM were 0.205 ± 0.013 μmole/ml-min and 0.016 ± 0.003 mmole/l., 0.0447 ± 0.009 μmole/ml-min and 0.018 ± 0.001 mmole/l., and 0.059 ± 0.007 μmole/ml-min and 0.020 ± 0.003 mmole/l. for choroid plexus (CP), hypothalamus (H), and caudate nucleus (CN), respectively. In CSF with added lead nitrate, the values were: CP, 0.101 ± 0.009 μmole/ml-min ($p<0.01$) and 0.005 ± 0.001 mmole/l. ($p<0.01$); H, 0.036 ± 0.008 μmole/ml-min ($p>0.05$) and 0.026 ± 0.008 mmole/l. ($p>0.05$); and CN, 0.046 ± 0.011 μmole/ml-min ($p>0.05$) and 0.025 ± 0.005 mmole/l. ($p>0.05$), respectively.

Thus, under *in vitro* conditions, lead may adversely and selectively affect a choroidal function. Studies are in progress to define more fully the effects of lead on tyrosine transport by CP and to determine whether similar effects occur with *in vivo* lead poisoning. Since tyrosine is a neurotransmitter precursor, these studies may be relevant to the diverse interactions of lead with central aminergic systems.

Cellular Response to Metals

Initial Action of Mercury at the Luminal Surface of an Alveolar Epithelium.

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An anuran lung lobe is a single alveolar sac which can be mounted in an Ussing chamber for the measurement of bioelectric properties and permeability. Functional comparisons with mammalian lungs suggest that the anuran lung may provide a useful assay system for the toxic effects of air pollutants on the alveolar epithelium. Previous studies of the excised bullfrog lung demonstrated a spontaneous electrical potential difference (ΔE) of 19 mV, pleura (serosa) positive, and a short-circuit current (I_{sc}) of 27 μa/cm². I_{sc} equalled active chloride secretion by a mechanism located at the luminal surface of the alveolar epithelium. Exposure of the lumen (mucosa) of the frog lung to solutions of $HgCl_2$ or amphotericin B (Am-B) induced an initial rise in ΔE and I_{sc} followed by a progressive decline. The increases after $HgCl_2$ were abolished by metabolic inhibitors but not by replacement of bathing solution ions. In contrast, the increases after Am-B were unaffected by metabolic inhibitors but disappeared when luminal Na^+ was replaced by choline or Mg^{2+}. After $HgCl_2$ the oxygen consumption Q_{O_2} of frog lung paralleled the initial increases in ΔE and I_{sc} but did not increase after Am-B. The Q_{O_2} of toad lung, which does not secrete Cl^- actively, was not increased by $HgCl_2$. Simultaneous tracer fluxes were measured on paired bullfrog lungs to assess the locus of $HgCl_2$ action. After mucosal $HgCl_2$ the serosal (S) to mucosal (M) radiochloride flux was selectively stimulated during the period of increased I_{sc}. When mucosal exposure of $HgCl_2$ was followed after 1 min by dimercaprol, the increase in I_{sc} was larger and prolonged; S to M chloride flux doubled whereas M to S chloride and both sodium fluxes were affected minimally. Mucosal Am-B caused initial, selective increases in sodium fluxes in both directions during the period of increased I_{sc}. The selective change in S to M chloride flux and metabolically dependent increases in bioelectric properties induced by $HgCl_2$ indicate an action at the apical surface of the alveolar epithelium on active chloride secretion. Since changes in bioelectric properties induced by Am-B do not require metabolic energy, the selective increase in sodium fluxes suggests that the raised ΔE and I_{sc} are the result of increased sodium entry across the apical surface.

Effects of Platinum Sulfate on Leucocyte and Platelet Metabolism, on Cytogenic Aberations, and on Immunologic Responses in the Rabbit.

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This research is a comprehensive study of biochemical, physiological, immunological, and mutagenic effects induced in blood leucocytes, blood platelets, and major organs of the mammalian body by exposure to platinum sulfate.

The IP LD_{50} for platinum sulfate in rabbits was found to be 210 mg/kg, with a range of 134 to 322 at the 95% confidence limits. Rabbits were injected daily with 2, 15, or 20 mg/kg of platinum sulfate IP for 1 or 3 weeks and then sacrificed. Others were allowed to recover for 1 week, 1 month, or 3 months before sacrifice. Saline control animals were included.

Weight loss occurred in rabbits receiving the two high doses. Platinum sulfate had no effect on rabbit skin irritation or guinea pig skin sensitization; however, it produced severe eve damage when left in the conjunctival sac for more than 30 sec.

No significant cytogenetic aberrations were observed in the leucocytes from rabbits treated with 20 mg/kg of platinum sulfate for up to 3 weeks. Significant cytogenetic aberrations occurred in human leucocytes exposed to 0.1 *mM* platinum sulfate (*in vitro*) in combination with 1 *mM* EMS. In human fibroblasts, platinum sulfate did not induce unscheduled DNA synthesis alone, but reduced by half the level of DNA repair induced by the positive control 4-nitroquinoline *N*-oxide. This finding supports the hypothesis that platinum may act as an intercalating agent inhibiting the repair of DNA damage caused by mutagenic or carcinogenic agents.

Leucocytes from platinum sulfate treated rabbits exhibited depressed RNA synthesis, DNA synthesis, and retarded protein and phospholipid turnover.

Mean corpuscular hemoglobin concentration and lymphocyte count were low, eosinophile count was markedly high. A functional anemia occurred after 1 week of treatment and persisted for at least 3 months.

At 15 and 20 mg/kg, platinum sulfate *in vivo* stimulated 5-HT uptake and β-glucuronidase activity, elevated aryl sulfatase, and altered phospholipid synthesis. These effects in rabbit platelets lasted 1 week to 3 months. No effect was observed in ADP-induced platelet aggregation.

Rabbit leucocytes incubated *in vitro* with 0.5*mM* platinum sulfate had depressed DNA, RNA, protein, and phospholipid biosynthesis. Platinum sulfate depressed RNA but not DNA synthesis in human leucocyte incubations.

In rabbit platelets, platinum sulfate, *in vitro*, depressed 5-HT uptake and release, and inositol incorporation. Choline incorporation was stimulated. ADP-induced aggregation and adenosine inhibition of aggregation were depressed. In human platelets, *in vitro*, 5-HT uptake was stimulated at low doses and depressed at high doses.

These studies suggest that high doses of platinum sulfate are inhibitory and low doses may be stimulatory to the metabolism of both leukocytes and platelets.

Primate Paneth Cell Degeneration Following Methylmercury Hydroxide Ingestion.

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The effects of methylmercury on the intestinal epithelium were studied in fourteen adolescent male *M. mulatta* monkeys weighing 3–5 kg. They were divided into three groups: two controls received daily applesauce vehicle without methylmercury; nine chronic low-dose animals received 0.2–1.0 mg methylmercury/day for 80 to 491 days; three acute high-dose animals received 2.0 mg methylmercury for 17–18 days, when they became terminally ill. Light and electron microscopic observations were made on samples of duodenum and ileum following perfusion and immersion fixation in a glutaraldehyde-paraformaldehyde fixative. Numerous uniquely structured inclusions were prominent in the Paneth cells of the chronic low dose animals and some necrotic Paneth cells were seen, especially in the most chronic and higher dosed animals of the group. Acute high dose produced some inclusions in the Paneth cells similar to that of the chronic low dose group but degenerative and necrotic cells were more frequently seen. These alterations were not seen in other intestinal epithelial cells. Paneth cells were selectively altered. These findings suggest that a function of Paneth cells may be to eliminate metals from the body.

Studies in the Relationships between Cadmium Chloride, Cadmium-Metallothienein and Cadmium-Induced Nephropathy.

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Cadmium-induced nephropathy occurs only after relatively long-term exposure to inorganic salts of cadmium ($CdCl_2$). Administered $CdCl_2$ initially accumulates in the liver followed by progressive increase in renal cadmium. Accumulation of Cd in liver and kidney is accompanied by increase in Cd binding protein, metallothionein. Cd–metallothionein (Cd–Mt) is thought to complex cadmium within cells and to provide protection against the potential cellular toxicity of cadmium. However, two recent groups of experiments in our laboratory suggest that Cd–Mt complex induced by exposure to $CdCl_2$ may also have a role in the pathogenesis of cadmium induced nephropathy.

In one group of experiments, Cd–Mt was isolated from livers of cadmium-exposed rats, labeled with ^{109}Cd, and administered IP to control rats. About 70% of ^{109}Cd-Mt was recovered from kidney and urine; less than 5% was present in liver. Within 12 hr, degenerative changes in the ultrastructure of renal tubular cells occurred. Fractionation of renal cells by differential centrifugation shows that within 2 hr after injection, 30% of Cd–Mt was in the nuclear fraction, 10% in the mitochondrial–lysosomal (mit–lys) fraction, and the remainder in the supernatant. After 24 hr, the amount in the nuclear and mit–lys fractions decreased.

Injection of an equal amount of $^{109}CdCl_2$ into control rats results in a similar organelle distribution of Cd in renal cells, but without abnormal structural or functional effect. Also, injection of ^{65}Zn–Mt had no abnormal effect.

In a second group of experiments, $CdCl_2$ (0.6 mg Cd/kg body weight per day, 5 days per week) was given to white male rats for 8 weeks. Glycosuria and the presence in the plasma of a chromatographic peak consistent with Cd-Mt appeared between 4 to 6 weeks of the experiment. Severe necrosis of proximal renal tubular lining cells was evident by week 7 or 8. Increase in urinary Cd did not occur until week 7 or 8.

These results suggest that the Cd–Mt complex *per se* is involved in the pathogenesis of tubular cell injury following cadmium exposure.

Role of Metallothionein in Tolerance to Cadmium Toxicity. A. PHILIP LEBER, *Battelle Columbus Laboratories, Columbus, Ohio 43201*, and T. S. MIYA, *Purdue University, West Lafayette, Indiana 47907.*

Mouse-derived metallothionein was found to consist of two distinct cadmium-binding proteins possessing similar absorption and metal-binding properties. A sensitive technique for analyzing proteins, pulse polarography, was successfully employed for the analysis of metallothionein. Mice exhibited tolerance to Cd toxicity after pretreatment with either Cd or Zn acetate. Cd doses of 0, 1.0, and 3.2 mg/kg, 48 hr prior to Cd challenge resulted in LD_{50} values of 4.5, 6.0, and 8.2 mg/kg, respectively. Levels of metallothionein increased with the pretreatment dose of cadmium.

In another experiment, the LD_{50} values for cadmium were 5.7 and 10.3 mg/kg for the control animals and animals administered 3 $\times$ 6.5 mg/kg of Zn at 12, 30, and 48-hr prior to a Cd challenge, respectively. Zn pre-treatment induced formation of a Zn-binding protein whose molecular weight appears to be identical to that of Cd-induced metallothionein. Cd-induced liver metallothionein contained Cd and Zn, while metallothionein resulting from Zn injections contained only Zn. Cd or Zn pretreated mice challenged with 10–12 mg Cd/kg 3 hr prior to sacrifice resulted in an *in vivo* displacement of Zn from both Cd– and Zn–metallothionein. This suggests that tolerance to Cd seen in pretreated mice is a result of the *in vivo* displacement of Zn from, and the subsequent chelation of Cd, to metallothionein. ^{14}C-Amino acid incorporation was found to be higher 12 hr after the administration of Cd than after 48 hr. The total amount of metallothionein protein in the liver at 48 hr was twice that found at 12 hr, while metallothionein-bound Cd was increased only slightly.

Effects of Cadmium Acetate on Microsomal Hemoproteins in Rat Liver. HARVEY C. KRASNY and DAVID J. HOLBROOK, JR., *Department of Biochemistry, School of Medicine, University of North Carolina, Chapel Hill, North Carolina 27514.*

Alterations in liver microsomal mixed function oxidase and in heme degradation were observed upon IP injection of male Sprague-Dawley rats with a single dose of cadmium acetate · $2H_2O$ (2.0 mg/kg, 7.5 μmole/kg). Three days after a pretreatment with Cd, the activities or contents (per mg microsomal protein) of aminopyrine demethylase, aniline hydroxylase, cytochrome P–450, and cytochrome b_5 were reduced by 47, 32, 44 and 27%, respectively. Seven days after a pretreatment, the above parameters were reduced by 37, 23, 19, and 15% respectively. Rats injected with ^3H-δ-aminolevulinic acid at 3 days after a Cd pretreatment showed an increased turnover in both the fast-phase and slow-phase fractions of the hemoprotein in CO-binding particles and a reduction in the ratio of fast-phase to slow-phase fractions. At 3 days after a pretreatment, a 2-fold increase occurred in microsomal heme oxygenase, the apparently rate-limiting enzyme (in normal liver) in degradative conversion of heme to bilirubin. This suggests a possible explanation for the increased heme turnover and reduced mixed function oxidase activity seen three days after Cd treatment. A partial recovery did occur, however, at 7 days after Cd treatment.

Transplacental Toxicity of Methylmercury to Fetal Rat Liver Mitochondria. BRUCE A. FOWLER and JAMES S. WOODS, *Environmental Toxicology Branch, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709.*

Marked ultrastructural changes have been previously reported in hepatocyte mitochondria from neonatal animals exposed to methylmercury during gestation. These results suggested alteration of normal mitochondrial biogenesis resulting from methylmercury exposure. The present study was undertaken to evaluate the impact of *in utero* exposure to methylmercury on the structure, function, and biogenesis of fetal liver mitochondria using correlative ultrastructural morphometric and biochemical techniques. Female rats were given access to deionized drinking water containing 0, 3, 5, or 10 ppm mercury as methylmercury hydroxide (MMH) for 4 weeks before being mated with untreated males. Pregnant animals were then continued on their respective treatments through day 19 of pregnancy, at which time they were killed and fetal liver processed for mercury analysis and ultrastructural and biochemical evaluation. Dose-related tissue concentrations of methylmercury were found in fetal liver of MMH-treated animals. Ultrastructural morphometric studies showed decreases of 18–25% in the volume density of fetal hepatocyte mitochondria, at the 5 to 10 ppm MMH dose levels. This finding was associated with decreased mitochondrial protein synthesis in the same groups of animals. Loss of respiratory control occurred in fetal mitochondria from animals in the 3 ppm MMH dose group while state 3 respiration could not be initiated in mitochondria from the 5 and 10 ppm MMH dose groups. Assays of the mitochondrial marker enzymes monoamine oxidase, cytochrome oxidase, δ-aminolevulinic acid synthetase which are localized on the

outer membrane, inner membrane, and loosely bound to the inner membrane, respectively were also performed. These studies disclosed dose-related decreases of up to 38, 22, and 32%, respectively, in the specific activities of these enzymes in MMH-treated animals. In contrast, malate dehydrogenase, a matrix marker enzyme, was unchanged at any dose level. Altered mitochondrial respiratory function and changes in membranal marker enzymes were found to persist in offspring of methyl mercury-treated mothers up to 12 weeks of age. These results suggest that the mitochondrial membrane may be a primary focal point of methylmercury activity and that *in utero* exposure to methylmercury may result in impaired postnatal development of mitochondrial function.

Tissue Lead Retention Following Administration of Equal Doses of Lead on Varying Dose Schedules in the Rat. KATHRYN R. MAHAFFEY, CHARLES L. STONE, and THOMAS A. BANKS, *Food and Drug Administration, Washington, D.C.*

The influence of concentration of lead (Pb) and frequency of exposure on tissue retention of Pb and on hematologic effects of Pb has been investigated. Forty-eight albino male rats of the Sprague-Dawley strain were fed nutritionally adequate, casein-based purified diets and various drinking solutions for a 90-day period. The drinking solutions administered were: group I, deionized water; group II, deionized water containing 200 ppm Pb as Pb acetate each day; group III, deionized water containing 600 ppm Pb every 3rd day and deionized water for the other 2 days; and group IV, deionized water containing 1800 Pb as Pb acetate every 9th day and deionized water for the other 8 days. Groups II, III, and IV were pair-watered to produce equal intakes of Pb at the end of the 90-day period. Analysis of the data by one-way analysis of variance and least significant difference techniques revealed no significant ($p>0.05$) differences in blood Pb between groups II, III, or IV. Kidney ($p< 0.05$) and femur (($p< 0.01$) Pb concentrations and urinary δ-aminolevulinic acid ($p< 0.05$) were significantly lower in animals receiving the 1800 μg Pb dose compared to either of the lower Pb doses. Groups II and III did not differ ($p> 0.05$) for these parameters. Free erythrocyte protoporphyrin and hematocrit did not differ significantly between groups II, III and IV. Regression analysis showed that femur Pb, kidney Pb, and urinary excretion of δ-aminolevulinic acid decreased significantly ($p< 0.05$) as the concentration of Pb in water increased and as the frequency of dosage decreased.

Over approximately a 10-fold range in dose frequency and dose concentration, blood Pb remained constant. Urinary excretion of δ-aminolevulinic acid was a better predictor of femur and kidney Pb concentration than was blood Pb. Reduction in kidney and femur Pb concentration in animals fed the highest concentration of Pb in water could be due to differences either in absorption or excretion of Pb. Increased excretion of Pb is thought to be the major reason for decreased kidney and femur Pb content at the 1800 μg Pb dosage. Barltrop and Meek (Postgrad. Med. J. 51: 805, 1975) report that kidney Pb concentrations in rats are directly proportional to dietary Pb over a 10-fold range in concentration.